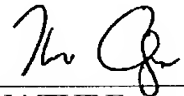


January 25, 2002

JC10 Rec'd PCT/PTO 25 JAN 2002

FORM PTO-1390 U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE		ATTORNEY'S DOCKET NUMBER
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371		GRUNP118
EXPRESS MAIL LABEL NO. EU000051594US		U.S. APPLICATION NO. To Be Assigned 10/049245
INTERNATIONAL APPLICATION NO. PCT/EP00/07206	INTERNATIONAL FILING DATE 26 July 1999 (26.07.1999)	PRIORITY DATE CLAIMED 26 July 2000 (26.07.2000)
TITLE OF INVENTION <b>A METHOD AND APPARATUS FOR THE SEPARATION OF PARTICLES, ESPECIALLY OF MACROMOLECULES, BY ELECTROPHORESIS</b>		
APPLICANT(S) FOR DO/EO/US Johan-Valentin KAHL; Julia NISSEN, Joachim RÄDLER, Roman ZANTL, Berenike MAIER, Ulf RÄDLER, Andreas HOHNER, and Reinhard GALNEDER		
Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:		
<ol style="list-style-type: none"> <li>1. <input checked="" type="checkbox"/> This is a <b>FIRST</b> submission of items concerning a filing under 35 U.S.C. 371.</li> <li>2. <input type="checkbox"/> This is a <b>SECOND</b> or <b>SUBSEQUENT</b> submission of items concerning a filing under 35 U.S.C. 371.</li> <li>3. <input type="checkbox"/> This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9), and (21) indicated below.</li> <li>4. <input checked="" type="checkbox"/> The US has been elected by the expiration of 19 months from the priority date (Article 31).</li> <li>5. <input checked="" type="checkbox"/> A copy of the International Application as filed (35 U.S.C. 371(c)(2)). <ol style="list-style-type: none"> <li>a. <input type="checkbox"/> is transmitted herewith (required only if not transmitted by the International Bureau).</li> <li>b. <input checked="" type="checkbox"/> has been transmitted by the International Bureau.</li> <li>c. <input type="checkbox"/> is not required, as the application was filed in the United States Receiving Office (RO/US).</li> </ol> </li> <li>6. <input checked="" type="checkbox"/> An English-language translation of the International Application as filed (35 U.S.C. 371(c)(2)). <ol style="list-style-type: none"> <li>a. <input checked="" type="checkbox"/> is transmitted herewith.</li> <li>b. <input type="checkbox"/> has been previously submitted under 35 U.S.C. 154(d)(4).</li> </ol> </li> <li>7. <input checked="" type="checkbox"/> Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3)). <ol style="list-style-type: none"> <li>a. <input type="checkbox"/> are transmitted herewith (required only if not transmitted by the International Bureau).</li> <li>b. <input type="checkbox"/> have been transmitted by the International Bureau.</li> <li>c. <input type="checkbox"/> have not been made; however, the time limit for making such amendments has NOT expired.</li> <li>d. <input checked="" type="checkbox"/> have not been made and will not be made.</li> </ol> </li> <li>8. <input type="checkbox"/> An English-language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).</li> <li>9. <input type="checkbox"/> An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).</li> <li>10. <input type="checkbox"/> An English-language translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).</li> </ol>		
Items 11 through 20 below concern document(s) or information included:		
<ol style="list-style-type: none"> <li>11. <input type="checkbox"/> An Information Disclosure Statement under 37 CFR 1.97 and 1.98.</li> <li>12. <input type="checkbox"/> An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.</li> <li>13. <input checked="" type="checkbox"/> A <b>FIRST</b> preliminary amendment.</li> <li>14. <input type="checkbox"/> A <b>SECOND</b> or <b>SUBSEQUENT</b> preliminary amendment.</li> <li>15. <input type="checkbox"/> A substitute specification.</li> <li>16. <input type="checkbox"/> A change of power of attorney and/or address letter.</li> <li>17. <input type="checkbox"/> A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 USC 1.821.</li> <li>18. <input type="checkbox"/> A second copy of the published international application under 35 USC 154(d)(4).</li> <li>19. <input type="checkbox"/> A second copy of the English-language translation of the international application under 35 USC 154(d)(4).</li> <li>20. <input checked="" type="checkbox"/> Other items or information:</li> </ol>		
3 Sheets of drawings – figs. 1 - 3		

JG13 Rec'd PCT/PTO 25 JAN 2002

U.S. APPLICATION NO. To Be Assigned <b>10/049245</b>		INTERNATIONAL APPLICATION NO. <b>PCT/EP00/07206</b>		ATTORNEY'S DOCKET NUMBER <b>GRUNP 118</b>	
21. <input checked="" type="checkbox"/> The following fees are submitted: <b>BASIC NATIONAL FEE (37 CFR 1.492(a)(1) - (5)):</b> Search Report has been prepared by the EPO or JPO ..... <b>\$890.00</b> International preliminary examination fee paid to USPTO (37 CFR 1.482) .... <b>\$690.00</b> No International preliminary examination fee paid to USPTO (37 CFR 1.482) But international search fee paid to USPTO (37 CFR 1.455(a)(2)) ..... <b>\$710.00</b> Neither international preliminary examination fee (37 CFR 1.482) nor International search fee (37 CFR 1.455(a)(2)) paid to USPTO ..... <b>\$1040.00</b> International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2) - (4) ..... <b>\$100.00</b>				<b>CALCULATIONS PTO USE ONLY</b>	
<b>ENTER APPROPRIATE BASIC FEE AMOUNT =</b>				<b>\$ 1040.00</b>	
Surcharge of \$130.00 for furnishing the oath or declaration later than <input type="checkbox"/> 20 <input checked="" type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(e))				<b>\$ 130.00</b>	
<b>CLAIMS</b>	<b>NUMBER FILED</b>	<b>NUMBER EXTRA</b>	<b>RATE</b>		
Total claims	46 - 20 =	26	x \$18.00	<b>\$ 468.00</b>	
Independent claims	5 - 3 =	2	x \$84.00	<b>\$ 168.00</b>	
<b>MULTIPLE DEPENDENT CLAIM(S) (IF APPLICABLE)</b>			+ \$260.00	<b>\$ 0.00</b>	
<b>TOTAL OF ABOVE CALCULATIONS =</b>				<b>\$ 1806.00</b>	
Reduction of 1/2 for filing by small entity, if applicable. Small Entity Status is Asserted.				<b>\$ 903.00</b>	
<b>SUBTOTAL =</b>				<b>\$ 0.00</b>	
Processing fee of \$130.00 for furnishing the English translation later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(f))				<b>\$ 0.00</b>	
<b>TOTAL NATIONAL FEE =</b>				<b>\$ 903.00</b>	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). <b>\$40.00</b> per property +				<b>\$ 0.00</b>	
<b>TOTAL FEES ENCLOSED =</b>				<b>\$ 903.00</b>	
				<b>Amount to be Refunded:</b>	<b>\$</b>
				<b>Charged:</b>	
A check in the amount of <b>\$ 903.00</b> in payment of the above fees is enclosed. Authorization is hereby given to charge any additional fees or deficiencies, or to credit any overpayment, to Deposit Account No. 501998.					
<b>NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.</b>					
SEND ALL CORRESPONDENCE TO:					
<p style="text-align: center;"><b>IP STRATEGIES, P.C.</b> 806 7<sup>th</sup> Street N.W. Suite 301 Washington, D.C. 20001 Telephone - (202) 289-2700 Facsimile - (202) 289-3594</p>					
				<p style="text-align: center;"> SIGNATURE NAME: Thomas M. Champagne REG. NO.: 36,478</p>	
Date: <u>January 25, 2002</u>					
Page 2 of 2					

10/049245

JC13 Rec'd PCT/PTO 25 JAN 2002

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Group Art Unit:  
Examiner:

In re Patent Application of:

Applicant : KAHL, et al. )  
U.S. Appln. No. : )  
International Appln. No. : PCT/EP00/07206 )  
International Filing Date : 26 July 1999 )  
Priority Date Claimed : 26 July 2000 )  
For : A METHOD AND APPARATUS )  
FOR THE SEPARATION OF )  
PARTICLES, ESPECIALLY OF )  
MACROMOLECULES, BY )  
ELECTROPHORESIS )  
Attorney Ref. : GRUNP118 )

PRELIMINARY  
AMENDMENT

Commissioner for Patents  
Washington, D.C. 20231

Sir:

Preliminary to examination, please amend the application as follows:

IN THE CLAIMS:

Please amend claim 15 to read as follows:

15. (Amended) A method according to claim 14, wherein a fluid lipid membrane, particularly comprising at least one of lipids activated by PEG and DAX-Chol

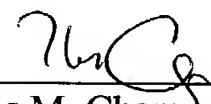
lipids are used as a substrate supported membrane, and wherein the exclusion area is provided by a non-fluid area of the fluid membrane.

**REMARKS**

Claim 15 is amended to eliminate a multiple dependency. Please enter the claim amendment prior to calculating the filing fee. Examination of the amended application is respectfully requested.

Respectfully submitted,

January 25, 2002  
Date

  
\_\_\_\_\_  
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15. (Amended) A method according to claim 14 [and claim 2], wherein a fluid lipid membrane, particularly comprising at least one of lipids activated by PEG and DAX-Chol lipids are used as a substrate supported membrane, and wherein the exclusion area is provided by a non-fluid area of the fluid membrane.

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**A method and apparatus for the separation of particles, especially of macromolecules, by electrophoresis.**

**Field of the Invention**

The present invention relates to a method and an apparatus for separating particles, in particular, macromolecules, such as DNA, RNA macromolecules, DNA, RNA oligomere and proteins by electrophoresis.

**Description of the Prior Art**

Methods using electrophoresis are preferably employed in molecular biology, genetic engineering and in the medical field to separate macromolecules having a different charge and a different size.

In the art substantially two different methods of the electrophoresis for the separation of macromolecules are known, namely the so-called gel electrophoresis and the capillary gel electrophoresis. In gel electrophoresis a gel applied to a plate is used; in capillary gel electrophoresis a gel-like polymeric solution in a capillary is provided.

In both the gel and capillary gel electrophoresis one takes advantage of the fact that macromolecules having a different charge and size migrate with a different velocity in a gel when influenced by an external electrical field, and bands are thereby generated each containing a species of the different macromolecules, i.e., the macromolecules having the same charge or the same size. The location of the bands is optically readout by color techniques or UV light absorption.

In order to readout the bands a layer of a coloring agent is applied to the gel and is maintained there at a precisely controlled temperature for a determined time. Thereafter the coloring reaction is prevented by withdrawing the water from the gel.

Typically, agarose, cellulose acetate or acrylamide are used as gels. Gel electrophoresis and capillary gel electrophoresis are used, for example, in DNA analysis in the medical

research and diagnosis. By means of restriction fragment analysis a prediction can be made about, for example, the genetic information, and for this reason gel electrophoresis has gained great importance for the diagnosis of genetically determined diseases.

Substantial disadvantages of methods using gel electrophoresis and capillary gel electrophoresis reside in the fact that, on the one hand, a significant amount of time is required for preparing and carrying out electrophoresis, and, on the other hand, that a large amount of sample material is required for the analysis.

In particular, in both methods the usual electrophoresis paths are relatively long. This entails migration times of approximately two hours. Furthermore, prior to the actual electrophoresis process, the gel has to be prepared, for example, it has to be heated. Automation of the this process is, in principle, possible is, however, accompanied by great effort, since the required equipment leads to high costs. Therefore, electrophoresis is often carried out manually, thereby additionally contributing to an increased time consumption.

Moreover, the conventional method may not arbitrarily be scaled down to, for example, reduce the amount of samples required.

In view of the above mentioned disadvantages, the present invention is directed to a method and apparatuses for carrying out a method in which, on the one hand, time consumption for the separation of macromolecules by means of electrophoresis may be reduced and which, on the other hand, are appropriate for scaling down so that already small amounts of samples are sufficient for the analysis.

### **Summary of the Invention**

The above-mentioned object is solved by a method for the electrophoretic separation of particles, in particular, of macromolecules, comprising applying the particles to be separated on a substrate-supported membrane such that the particles are mobile across the surface of the substrate-supported membrane. The method further comprises providing an electrical field such that directions of the electrical field are oriented along the surface across which the particles are mobile. Additionally, the method includes

temporarily modifying the electrical field and/or using a substrate-supported membrane having a structured surface, wherein the electrical field is temporarily modified and/or the substrate-supported membrane is structured in such a manner that a force is acting on the particles which leads to a movement depending on the length of the particles.

By means of a defined selection of the applied electrical field and/or a corresponding structuring of the membrane, a force acts on the particles that depends on the length of the macromolecules, and consequently a different path length in the electrical field is obtained for macromolecules depending on their size. Contrary to the prior art, in the present method, the running times may be significantly reduced in that the macromolecules no longer have to propagate in the gel, but instead are bound at the surface of the membrane, yet are otherwise freely movable. The high degree of mobility of the macromolecules on the membrane, therefore, leads to a significant reduction of the time period necessary to carry out electrophoresis. Furthermore, time consumption for the preparation of a membrane is remarkably reduced compared to the time consumption required for casting a gel.

In a further preferred embodiment of the present invention, a fluid lipid membrane is used as the substrate-supported membrane. By using such a membrane, it is insured that, on the one hand, the particles are bound to the membrane and, on the other hand, the particles have a sufficient degree of mobility on the surface of the membrane. The three-dimensional movement known from the prior art may accordingly be reduced to a quasi-two-dimensional movement. Such membranes may comprise, for example, PEG functionalized lipids or DAC-Chol: 3-beta-(N-(N,N'-Dimethylaminoethan)-carbonyl) (-cholesterolhydrochlorid). Preferably, a cationic fluid lipid membrane may be used for this purpose. By means of such a membrane typically negatively charged DNA, RNA macromolecules and DNA, RNA oligomers, respectively, may be bound to the membrane.

According to a further embodiment amphiphilic macromolecules may be used for building up the membrane. Amphiphilic macromolecules are characterized in that they form monolayers and bilayers in an aqueous solution.



According to a further embodiment of the previous method, a fluid lipid membrane may be used that comprises monolayers or bilayers including charged lipids. Accordingly, on the one hand, good adherence of the membrane to the substrate is guaranteed and, on the other hand, binding of the macromolecules to the membrane is insured. Moreover, in this way, the membrane may be formed relatively thin so that the observation of the bands with optical means is possible without any problems.

According to a first alternative of the above described method, a pulsed electrical field is used. The macromolecules of different size that have been applied to the membrane initially perform an unordered Brownian movement. If then a pulsed electrical field is applied, the various macromolecules are oriented along the lines of the electrical flux during the first pulse. This orientation occurs depending on the size of the macromolecules, wherein the smaller macromolecules are aligned more rapidly than are the larger ones. The macromolecule starts its movement in the direction of a field gradient once the macromolecule is oriented correspondingly to the electrical field. As a consequence, smaller macromolecules start moving in the electrical field earlier than larger macromolecules. During the time period between the first and the second pulse, again an unordered Brownian movement of the macromolecules occurs; however, due to the greater migration distance the smaller macromolecules have moved during the first pulse, they perform their unordered movement at a different location than do the larger macromolecules. If now the second pulse is applied, again, orientation of the macromolecules along the field line takes place. Again, smaller macromolecules are aligned more rapidly than larger macromolecules and again move a larger distance in the electrical field during the second pulse. As a result, in this way a separation of the macromolecules can be achieved depending on their size.

According to a second alternative of the above described method, an alternating field can be used on which a time constant field is superposed. The macromolecules substantially moved along the lines of electrical fluxes driven by the time constant field. However, the movement in this field is also influenced by the alternating field in which the macromolecules are oriented. Since, analogous to the above case, the macromolecules are oriented in the alternating field with a rate depending on their size and also move in the alternating field, a smaller macromolecule again moves a larger distance per time unit. In that the macromolecules are not only oriented under the influence of the



movement taking place during the electrophoresis are defined. This results in an improved resolution of the method.

When using a fluidal membrane, the exclusion area may be realized by a non fluid area in the fluid membrane. This non-fluid area may be obtained, for example, in that the substrate is coated with a material on which the actual fluid membrane is not fluid. For example,  $\text{Al}_2\text{O}_3$  is appropriate for this purpose. Such an exclusion area can also be obtained in that a different material is applied to the substrate so that in this area no fluid membrane can form. For instance,  $\text{Al}_2\text{O}_3$  is suitable for this purpose.

The above described methods can be employed in a method for observing an electrophoretic separation. For this purpose, preferably, digitized image data are recorded by, for example, a video camera coupled to an optical microscope while performing the electrophoretic separation. Thereafter, these recorded image data may be evaluated by using a computer.

Compared to the prior art, this embodiment offers the large advantage that also dynamic processes may be observed in a simple manner. Accordingly, it is possible, for example, to observe DNA-cutting enzymes during their activity.

The above described methods for the electrophoretic separation are, as previously explained, especially suited for separation of DNA, RNA macromolecules, DNA, RNA oligomers. Additionally, with this method a plurality of other macromolecules, such as proteins, may also be investigated.

Advantageously the so-called iso-electrical focusing may be used to improve the resolution. Hereby, the particles migrate in a pH gradient up to a pH value corresponding to their iso-electrical point at which they exhibit a zero net charge. That is, the migration speed at this point is also zero. In the one-dimensional protein electrophoresis the pH gradient field is parallel to the electrical field. In the two-dimensional electrophoresis the pH gradient is perpendicular to the applied field.

According to the present invention, a substrate is provided in particular for supporting the membrane during the performing of one of the above described methods, which is

comprised of an optical transparent material. With such a substrate it is possible, especially in combination with the relatively thin membrane, to directly observe or to record, using a video camera, the bands of the different sizes of the macromolecules, which have formed after the electrophoretic separation. This provides the advantage that the macromolecules may be maintained in their native state during analysis, thereby allowing the macromolecules to be available for analysis steps to be performed later on, such as a DNA hybridization.

According to a further embodiment the substrate may comprise glass as an optically transparent material.

Alternatively, plastics may also be used as an optically transparent material. In particular, plastics such as PC, PMMA, PS, PE or plastics of cyclic olefins may also be used for this purpose. Since plastics may be processed more simply than glass, plastics are especially suited when the substrate shall include complex structures, for example for the performing of the method with a structured substrate.

In the method in which a structured substrate is employed especially a substrate is appropriate that includes a surface having ribs formed therein. Preferably, the ribs and the substrate exhibit a periodicity ranging from 2nm to 200nm. As a height of the ribs, a height ranging from 0.1nm to 10nm has proven to be suitable.

The essential point of structured substrate resides in the fact that macromolecules provided on a membrane that is applied on such a substrate experience a frictional force that depends on the size of the macromolecules. Therefore, differently formed structures leading to this effect may be used.

Although these substrates may advantageously be used in the above described methods, these substrates may also be employed in other applications, such as in expanding or orienting of macromolecules. This is especially true for the substrates having a structured surface such as the rib-shaped surface.

Moreover, according to the present invention a substrate supported membrane is provided that is especially suited for performing the above described methods and which

comprises one of the previously described substrates and a fluid lipid membrane applied on the corresponding substrate.

By means of this substrate supported membrane simple means are provided for the carrying out of the above described methods.

Advantageously, the substrate supported membrane may comprise a fluid lipid membrane having cationic lipids. Advantageously amphiphilic macromolecules may also be used for forming the membrane. Furthermore, the fluid lipid membrane may comprise bilayers of charged lipids. Moreover, the fluid membrane of the substrate supported membrane may comprise at least one non-fluid area.

With these advantageous embodiments the advantages may be attained that have been discussed with respect to the methods. To avoid any repetitions it is referred to the corresponding parts of the description of the present methods.

According to a further embodiment the fluid lipid membrane of the substrate supported membrane may be dried up. Therefore, the substrate having the membrane dried up may be stored. For usage the substrate supported membrane has only to be swelled in water and/or a buffer solution. With membranes prepared in such a manner possible errors and deviations in producing the membrane, in particular in preparing the solutions for the membrane, may be avoided, so that the reproducibility of results is increased.

Moreover, in accordance with the present invention, there is additionally provided a microchannel electrophoresis chamber comprising at least one channel the bottom surface of which includes one of the previously described substrates wherein the microchannel chamber comprises an electrode assembly.

In such a microchannel electrophoresis chamber the various process steps such as determination of the start point, the electrophoretic separation of particles by the movement in an electrical field and the optical evaluation of the bands may be integrated. Consequently, the present method is significantly simplified compared to the prior art technique that required to carry out these process steps in different stages and in different work places. Moreover, in such a microchannel electrophoresis chamber it is

possible to expand, to manipulate and to simultaneously make macromolecules available for optical methods.

Preferably, hereby each channel has a width in the range from 1 micrometer to 10 millimeters and depth in the range from 1  $\mu\text{m}$  to 5000  $\mu\text{m}$ .

According to a preferred embodiment the microchannel electrophoresis chamber may comprise a plurality of channels arranged in the form of a two-dimensional matrix. It is thus possible to carry out a plurality of experiments at the same time.

According to a preferred embodiment of the microchannel electrophoresis chamber the electrode assembly may comprise two electrodes that are provided at the respective longitudinal ends of each channel. This embodiment leads to a further simplification of the experimental set up. Hereby, the electrodes may already be integrated in the microchannel electrophoresis chamber and the microchannel electrophoresis channel has merely to be connected at the measurement location. Moreover, since the electrodes are already provided in the microchannel electrophoretic chamber, a predetermined arrangement with respect to each channel and therefore also with respect to the membrane may be realized, thereby resulting in a fixedly defined field with respect to the membrane. Thereby, a further scaling-down may be obtained compared to the prior art, since in the prior art the scaling-down is limited due to the position accuracy of the electrical field with respect to the membrane.

According to a further embodiment the electrode assembly may alternatively or additionally comprise two electrodes extending longitudinally along the channel direction at both sides of each channel. This embodiment is especially appropriate for the above described method in which an alternating field and a time constant field provided in a crossed manner thereto is used. It is to be understood that also in this case the advantages may be obtained that have been discussed with reference to the above described electrode assembly.

In the above described microchannel electrophoresis chamber the substrate may be coated with a fluid lipid membrane. This fluid lipid membrane may comprise cationic lipids. Advantageously, the fluid lipid membrane may comprise amphiphilic

macromolecules. Moreover, the fluid lipid membrane may include bilayers of charged lipids, the fluid lipid membrane may be provided in a dried form and/or may at least comprise a non-fluid area.

By these special embodiments the advantages already discussed with reference to the various methods and the various substrate supported membranes may also be attained. In order to avoid any redundancies it is merely referred to the corresponding explanations of these features with respect to the methods and the substrate supported membranes.

### **Brief description of the drawings**

In the following the present invention will be describes with reference to various embodiments by referring to the accompanying drawings in which:

Figure 1: depicts an embodiment of a microchannel electrophoresis chamber for explaining a first embodiment of the method for the electrophoretic separation of particles in accordance with the present invention;

Figure 2: shows a first embodiment of a substrate supported membrane for explaining a second embodiment of the method for the electrophoretic separation of particles according to the present invention; and

Figure 3: depicts a second embodiment of a substrate supported membrane for explaining a third embodiment of the method for the electrophoretic separation of particles according to the present invention.

Figure 1 depicts an embodiment of a microchannel electrophoresis chamber for explaining a first embodiment of the method for the electrophoretic separation of particles according to the present invention. A microchannel electrophoresis chamber 1 comprises a chamber body 2, in which a channel 3 is provided. The bottom surface of channel 3 is hereby provided in form of a non-structured substrate 4. The substrate 4 is optically transparent comprising, for example, glass, plastics such as PMMA, PC, PS, PE or similar materials. Moreover, the substrate is thinned down to approximately 100µm.

Because of this, the microchannel electrophoresis chamber 1 is directly available for optical read out methods.

The substrate 4 may be inserted in a corresponding opening in the chamber body 2 or as in the present case, may directly be formed as a part of the chamber body 2. To this end, a channel having a corresponding depth in a range of 1µm to 5000µm may be provided in the chamber body.

A fluid membrane 5 is applied on the substrate as is especially depicted in the enlarged view of Figure 1. In the present embodiment, the membrane 5 is provided in form of a bilayer lipid membrane, such as a DPOC/DOTAP membrane.

Moreover, the electrophoresis chamber comprises an electrode assembly 6 including two electrodes provided at the longitudinal ends of the channel 3, wherein the electrodes comprise conventional materials such as platinum, gold, Ag/AgCl and the like. One of these electrodes is grounded, whereas a pulsed voltage in a range of 2 to 1000V is applied to the other electrode, as will be explained in the following.

As is further depicted in the enlarged view, two macromolecules are provided on the membrane 5.

The entire arrangement depicted in Figure 1, i.e. the membrane 5, the macromolecules 7 and the substrate 4 are within a liquid, for example water or a molecule-stabilizing buffer.

In the following a first embodiment of the method for the electrophoretic separation of macromolecules is described with reference to Figure 1.

In the embodiment depicted in Figure 1 and as also indicated in Figure 1, an electrical field is used. The macromolecules 7 of different size that have been applied on the membrane 5 perform an unordered Brownian movement between the voltage pulses, that is, at a voltage of 0V. During the voltage pulses, however, the various macromolecules are initially aligned along the lines of electrical fluxes. This alignment or orientation occurs depending on the size of the macromolecules, wherein the smaller macromolecules are oriented more rapidly than the larger ones. After a macromolecule is



oriented or aligned in the electrical field, it also starts moving in the electrical field during the voltage pulses and it moves in the direction of the electrical field. Since smaller macromolecules are oriented in the electrical field more rapidly than larger macromolecules, the smaller macromolecules start moving in the electrical field earlier and therefore move the larger distance in the field during the voltage pulses than the larger macromolecules.

During the application of a pulsed voltage the macromolecules therefore move, depending on their charge and size respectively, over different distances on the membrane 5 in the channel 3 and they collect in so-called bands 8 according to their charge or size as is depicted in Figure 1.

Figure 2 shows a first embodiment of a substrate supported membrane 10 for explaining a second embodiment of the method for the electrophoretic separation of particles according to the present invention. In the following, merely the differences between both arrangements are described so as to avoid any repetition. Hereby, the same numerals indicate the same components of the corresponding arrangement.

The substrate supported membrane 10 in Figure 2 differs from the arrangement depicted in Figure 1 substantially by the electrode assembly. Moreover, the substrate supported membrane 10 is provided in a chamber body of the channel floor. The remaining components correspond to those of the structure depicted in Figure 1; for the description thereof, it is therefore referred to the corresponding discussion provided above.

The electrode assembly of the substrate supported membrane depicted in Figure 2 comprises two electrodes 6a and two electrodes 6b. A constant voltage (DC) which preferably ranges from 2 to 1000V is applied to the electrodes 6a and an alternating voltage (AC) preferably in the range of 2 to 1000V and having a frequency in the range of 0.1 to 200Hz is applied to the electrodes 6b.

As in the arrangement shown in Figure 1, also in this case the membrane, the substrate and the macromolecules are provided in a liquid, for example in water.

In the following, a second embodiment of the method for the electrophoretic separation of macromolecules by using a substrate supported membrane as shown in Figure 2 will be described.

By means of the time constant field caused by the electrodes 6a, the macromolecules move essentially in the direction of the field gradient. The motion in this field is however also influenced by the alternating field created by the electrodes 6b, in which the macromolecules are oriented and also move. Since the macromolecules, analogous to the above case, are oriented and move in the alternating field depending on the size of the macromolecules, the smaller macromolecules again move a larger distance per time unit. Since the macromolecules not only are oriented under the influence of the alternating field, but also move in the alternating field, they perform a zig-zag movement on the surface of the membrane as is indicated in Figure 2.

As in the first method, the macromolecules also gather in the bands 8, which are indicated on the membrane on Figure 2 and which are additionally presented in a histogram (the histogram depicts the number of molecules versus the distance moved on the substrate supported membrane), since the macromolecules move different distances according to the size and charge, respectively.

Figure 3 depicts a second embodiment of a substrate supported membrane for explaining a third embodiment of the method for the electrophoretic separation of particles according to the present invention.

This embodiment differs from the substrate supported membrane shown in Figure 2 in that the surface of the substrate 4 is provided with ribs 9. The remaining arrangement of the embodiment corresponds to that depicted in Figure 2, and therefore it is referred to the explanation of the latter embodiment to avoid any repetition

In the embodiments of Figure 3 a substrate is used the ribs of which exhibit a periodicity in the range of 2 nm to 200 nm. The height of the ribs thereby ranges from 0.1 nm to 10 nm. In a substrate comprising the above indicated plastics, the structuring can be formed by, for example, imprinting a dye containing the negative of the desired form. To this end,

the plastic is preferably heated. A silicon wafer cut in the (111) plane and etched with KOH may be used as the dye.

By employing the substrate 4 having the ribs 9, the movement and, in particular, the orientation in the electrical field of the macromolecules, depending on the size of the macromolecules, on a membrane applied on a such a substrate is influenced even more intensely. Especially, the frictional force for larger macromolecules during their orientation is increased more intensely than that for smaller macromolecules leading, in total, to a further discrimination with respect to the size of the macromolecules.

For a further explanation of the present invention, various examples will be described which have been performed with the above described means.

#### **Example 1:**

##### Preparation of a substrate supported membrane by vesicular fusion

In example 1 the substrate supported membrane was realized by manufacturing of a cationic bilayer of lipids by vesicular fusion of a glass support. To this end, for example, lipids solved in chloroform were mixed. A typical ratio of DOPC/DOTAP is 9/1. This solution was dried up and then the lipids were swelled with water or a buffer solution, such as HEPES 10mM, NaCL 10mM, EDTA 1mM. The lipid concentration was approximately 1mg/ml. This solution was exposed to sonic waves with a blow pipe sonic wave means for 1 to 2 minutes. The surface to be coated was heated prior to the actual vesicular fusion process to be greatly hydrophilic. Suitable for these purposes is a treatment of 1 minute with a 5M KOH solution. For the filling of a chamber, as previously described, a vesicular solution of approximately 2ml was provided to the chamber. After approximately 2 hours, the chamber was thoroughly purged with a buffer solution to remove the excess lipid. After this treatment, a bilayer having a thickness of approximately 4 nm remained in the chamber. Between the bilayer and the substrate, an approximately 0.1 nm thick water layer was formed. In this example, the membrane had a lateral self diffusion constant of approximately  $1 \mu\text{m}^2/\text{s}$ .

Since, in the present example, the electrophoretic separation should be evaluated by microscopy with high resolution, a transparent optically inert substrate or a chamber floor was used, the thickness of which was between 100 and 200  $\mu\text{m}$ .

#### Separation of the DNA by means of pulsed fields

The macromolecules to be separated, for example, DNA 80 bp (base pairs) and 40 bp were also inserted into the chamber.

Double strand DNA of the length of 80 bp exhibited a self diffusion constant of  $0.2 \mu\text{m}^2/\text{s}$  on the fluid membrane of the present example, whereas DNA having a length of 40 bp exhibited a diffusion constant of  $0.4 \mu\text{m}^2/\text{s}$ .

By the application of a pulsed field between 0.1V/cm and 100V/cm, the DNA macromolecules separate. Advantage was taken from the fact that the DNA oligomers were randomly oriented by self-rotation diffusion after each turn off of the field. This effect breaks up the proportional ratio between the number of DNA base pairs and the mobility.

To avoid any polarization of the electrodes, platinum electrodes or Ag/AgCl electrodes that had been blackened were used. Depending on the chamber size, salt contents of the buffer solution and the applied voltage, a current between 0.1  $\mu\text{A}$  and 10  $\mu\text{A}$  is flowing. The electrical field was applied for approximately 10 minutes.

#### Display of the DNA bands by fluorescence marking

By adding a DNA colorant, for example TOTO with a ratio of one TOT macromolecule to five DNA base pairs, the DNA is fluorescence-marked after approximately 10 minutes at room temperature. To this end, water/buffer containing the corresponding amount of TOTO is purged into the chamber. For observing the bands, and Axiovert 100 of the Carl Zeiss company was used. The two bands of the oligomers having a length of 40 bp and 80 bp respectively were observable.

#### **Example 2:**

### Preparation of a cationic monolayer or bilayer by means of a Langmuir-Blodgett technique

By means of the Langmuir-Blodgett technique, a fluid membrane was applied to PMMA; instead of PMMA, also PC, PE, PS, PVA as well as cyclic olefins were used. In the Langmuir-Blodgett technique, amphiphilic molecules solved in chloroform are spread on the water surface of a trough made of teflon so that the hydrophilic portion of the molecule penetrates the water and the hydrophobic part projects from the water. This system can be compressed to the desired lateral pressure by means of an appropriate barrier. If now a vertically oriented platelet made of the above-mentioned materials is dipped into the water through this film, a fluid monolayer forms on the surface of the platelet.

The same experiments as in example 1 were carried out with this substrate supported membrane and the same results were obtained.

#### **Example 3:**

A substrate supported membrane according to example 1 or example 2 was prepared.

### Separation of the DNA by cross fields

Contrary to the first and second examples, cross fields, i.e. a constant field superimposed by an alternating field instead of a pulsed field was applied for the separation of the DNA 80 bp and the DNA 40 bp.

To this end, the constant field exhibited a field strength between 2 and 200V/cm, the AC voltage field had a field strength between 2 and 200V/cm at a frequency from 0.1 to 100 Hz. Thereby, platinum/platinum black or alternatively Ag/AgCl electrodes were used.

The same experiments as in example 1 were performed with the substrate supported membrane and the same results were obtained.

### Example 4:

A substrate supported membrane according to Example 1 or Example 2 was prepared

### Separation of the DNA by structuring the substrate

The membrane is applied to a previously structured substrate as is shown, for example, in Figure 3 and is described with reference to this Figure. This substrate comprised ripple marks or a roughness having a height of approximately 0.1 nm and a periodicity of about 100 nm. Moreover, the used substrates were transparent and had a thickness from 100  $\mu\text{m}$  to 200  $\mu\text{m}$ . The electrode assembly was driven as in Example 3.

The results received with this arrangement correspond to the result obtained in Example 3, wherein the relative distance between the bands was larger.

### Example 5:

### Collecting the DNS on a line by providing non-fluid areas

Parts of the chamber floor were coated with materials, on which the applied membrane is non-fluid, as is described in the Examples 1 – 4. To this end, for example  $\text{Al}_2\text{O}_3\text{P}$  or PMMA were used. These areas represent an exclusion area for the DNA macromolecules that are mobile on the membrane. By applying an electrical field forcing the DNA in the direction of the exclusion area, the DNA macromolecules can be collected at the boundary between the membrane and the exclusion area.

By means of this membrane, and electrophoretic separation as in Example 1 was performed. In the result, sharper bands were observed, i.e. bands having an extension in the direction of the field that is less than the extension of the bands obtained in Example 1.

### Example 6:

Collecting the DNS on a line by providing areas that are not wetted by the membrane



## Claims

1. A method for the electrophoretic separation of particles, particularly of membrane-adherent macromolecules, the method comprising:

applying the particles to be separated on a substrate supported membrane such that the particles are mobile across the surface of the substrate supported membrane;

providing an electrical field such that the direction of the field is oriented along the surface across which the particles are mobile;

performing at least one of:

temporarily modifying one of the strengths and the direction of the electrical field such that a force is acting on the particles that leads to a movement depending on the length of the particles and

using a substrate supported membrane having a structured surface, wherein the substrate supported membrane is structured such that a force is acting on the particles that leads to a movement depending on the length of the particles.

2. A method according to claim 1, wherein a fluid lipid membrane, particularly comprising at least one of lipids activated by PEG and DAX-Chol lipids are used as a substrate supported membrane.
3. A method according to claim 2, wherein a cationic fluid lipid membrane is used.
4. A method according to claim 2, wherein a fluid membrane is used that comprises amphiphilic macromolecules.
5. A method according to claim 2, wherein a fluid lipid membrane is used that comprises bilayers of charged lipids.



6. A method according to claim 1, wherein a pulsed electrical field is used.
7. A method according to claim 1, wherein an alternating field is used on which a time constant field is superimposed.
8. A method according to claim 7, wherein the alternating field and the time constant field are superimposed in a crosswise manner.
9. A method according to claim 1, wherein a substrate having a surface comprising ribs is used for supporting the membrane.
10. A method according to claim 9, wherein a substrate is used that exhibits a periodicity ranging from 2 nm to 200 nm.
11. A method according to claim 9, wherein a substrate is used in which the height of the ribs is in the range of 0.1 nm to 10 nm.
12. A method according to claim 9, wherein a time constant field is used, the direction of which is substantially parallel to the ribs.
13. A method according to claim 1, wherein said movement is a rotation.
14. A method according to claim 1, wherein the membrane comprises an exclusion area in which the particles are not mobile and wherein prior to performing the electrophoresis, the particles are collected at said exclusion area upon the application of an electrical field.
15. A method according to claim 14 and claim 2, wherein the exclusion area is provided by a non-fluid area of the fluid membrane.
16. A method of observing an electrophoretic separation, the electrophoretic separation comprising applying the particles to be separated on a substrate

supported membrane such that the particles are mobile across the surface of the substrate supported membrane;

providing an electrical field such that the direction of the field is oriented along the surface across which the particles are mobile;

performing at least one of:

temporarily modifying one of the strengths and the direction of the electrical field such that a force is acting on the particles that leads to a movement depending on the length of the particles and

using a substrate supported membrane having a structured surface, wherein the substrate supported membrane is structures such that a forces is acting on the particles that leads to a movement depending on the length of the particles.

wherein the method further comprises:

recording digitized image data from the electrophoretic separation; and

evaluating the recorded image data by a computer.

17. A method according to claim 1, wherein the particles to be separated comprise at least one of DNA, RNA, DNA-, RNA-oligomers and proteins.
18. A method according to claim 1, wherein the particles migrate in a pH gradient.
19. A method according to claim 18, wherein the pH gradient is provided in a parallel manner with respect to the electrical field.
20. A method according to claim 18, wherein the pH gradient is provided substantially perpendicularly to the electrical field.

21. A substrate, especially for supporting a membrane for carrying out of a method comprising: applying the particles to be separated on a substrate supported membrane such that the particles are mobile across the surface of the substrate supported membrane;

providing an electrical field such that the direction of the field is oriented along the surface across which the particles are mobile;

performing at least one of:

temporarily modifying one of the strengths and the direction of the electrical field such that a force is acting on the particles that leads to a movement depending on the length of the particles and

using a substrate supported membrane having a structured surface, wherein the substrate supported membrane is structures such that a forces is acting on the particles that leads to a movement depending on the length of the particles;

the substrate comprising an optically transparent material.

22. A substrate according to claim 21, wherein the substrate includes glass as the optically transparent material.
23. A substrate according to claim 21, wherein the substrate comprises plastics as the optically transparent material.
24. A substrate according to claim 23, wherein the plastics comprises one PC, PMMA, PS, PE and plastics formed of cyclic olefins.
25. A substrate according to claim 21, wherein a surface of the substrate is structured.
26. A substrate according to claim 25, wherein the surface comprises ribs.

27. A substrate according to claim 26, wherein the ribs exhibit a periodicity ranging from 2 nm to 200 nm.
28. A substrate according to claim 26, wherein the ribs have a height ranging from 0.1 nm to 10 nm.
29. A substrate supported membrane, particularly for performing a method comprising:
- applying the particles to be separated on a substrate supported membrane such that the particles are mobile across the surface of the substrate supported membrane;
  - providing an electrical field such that the direction of the field is oriented along the surface across which the particles are mobile;
  - performing at least one of:
    - temporarily modifying one of the strengths and the direction of the electrical field such that a force is acting on the particles that leads to a movement depending on the length of the particles and
    - using a substrate supported membrane having a structured surface, wherein the substrate supported membrane is structured such that a force is acting on the particles that leads to a movement depending on the length of the particles;
- the substrate comprising:
- a substrate including an optically transparent material; and
  - a fluid lipid membrane.

30. A substrate supported membrane according to claim 29, wherein the fluid lipid membrane comprises cationic lipids.
31. A substrate supported membrane according to claim 29, wherein the fluid lipid membrane comprises amphiphilic macromolecules.
32. A substrate supported membrane according to claim 29, wherein the fluid lipid membrane comprises bilayers of charges lipids.
33. A substrate supported membrane according to claim 29, wherein the fluid lipid membrane is dried up.
34. A substrate supported membrane according to claim 29, wherein the fluid membrane comprises at least one fluid area.
35. A microchannel electrophoresis chamber comprising:  
  
at least one channel having a bottom surface including a substrate that comprises an optically transparent material; and  
  
an electrode assembly.
36. A microchannel electrophoresis chamber according to claim 35, wherein each channel comprises a width ranging from 1  $\mu\text{m}$  to 10 mm.
37. A microchannel electrophoresis chamber according to claim 35, wherein each channel comprises a depth ranging from 10 nm to 20  $\mu\text{m}$ .
38. A microchannel electrophoresis chamber according to claim 35, wherein a plurality of channels is provided that is arranged in form of a two-dimensional matrix.

39. A microchannel electrophoresis chamber according to claim 35, wherein the electrode assembly comprises two electrodes provided at each longitudinal end of each of the channels.
40. A microchannel electrophoresis chamber according to claim 35, wherein the electrode assembly comprises two electrodes longitudinally expanding in the direction of the channel at both sides of each channel.
41. A microchannel electrophoresis chamber according to claim 35, wherein the substrate is coated with a fluid lipid membrane.
42. A microchannel electrophoresis chamber according to claim 41, wherein the fluid lipid membrane comprises cationic lipids.
43. A microchannel electrophoresis chamber according to claim 41, wherein the fluid lipid membrane comprises amphiphilic macromolecules.
44. A microchannel electrophoresis chamber according to claim 41, wherein the fluid lipid membrane comprises bilayers of charged lipids.
45. A microchannel electrophoresis chamber according to claim 41, wherein the fluid lipid membrane is dried up.
46. A microchannel electrophoresis chamber according to claim 41, wherein the fluid membrane comprises at least one non-fluid area.

**Abstract**

The present invention provides a method for the electrophoretic separation of particles, especially of macromolecules, comprising the steps of applying the particles to be separated on a substrate supported membrane, such that the particles are mobile across the surface of the substrate supported membrane; providing an electrical field such that electrical fields are formed along the surface across which the particles are mobile; and temporarily modifying the electrical field and/or adding a substrate supported membrane having a structured surface, wherein the direction and/or the strength of the electrical field are temporarily modified and/or wherein the substrate supported membrane is structured so that a force is acting on the particles that leads to a movement depending on the length of the particles. Moreover, a substrate is provided, in particular, for supporting a membrane during the performing of the inventive methods, that comprises an optically transparent material. Moreover, the invention provides a substrate supported membrane for carrying out the inventive methods including an inventive substrate as defined in the claims and a fluid lipid membrane. Moreover, the present invention provides a microchannel electrophoresis chamber having at least one channel with a bottom surface including an inventive substrate, and an electrode assembly.

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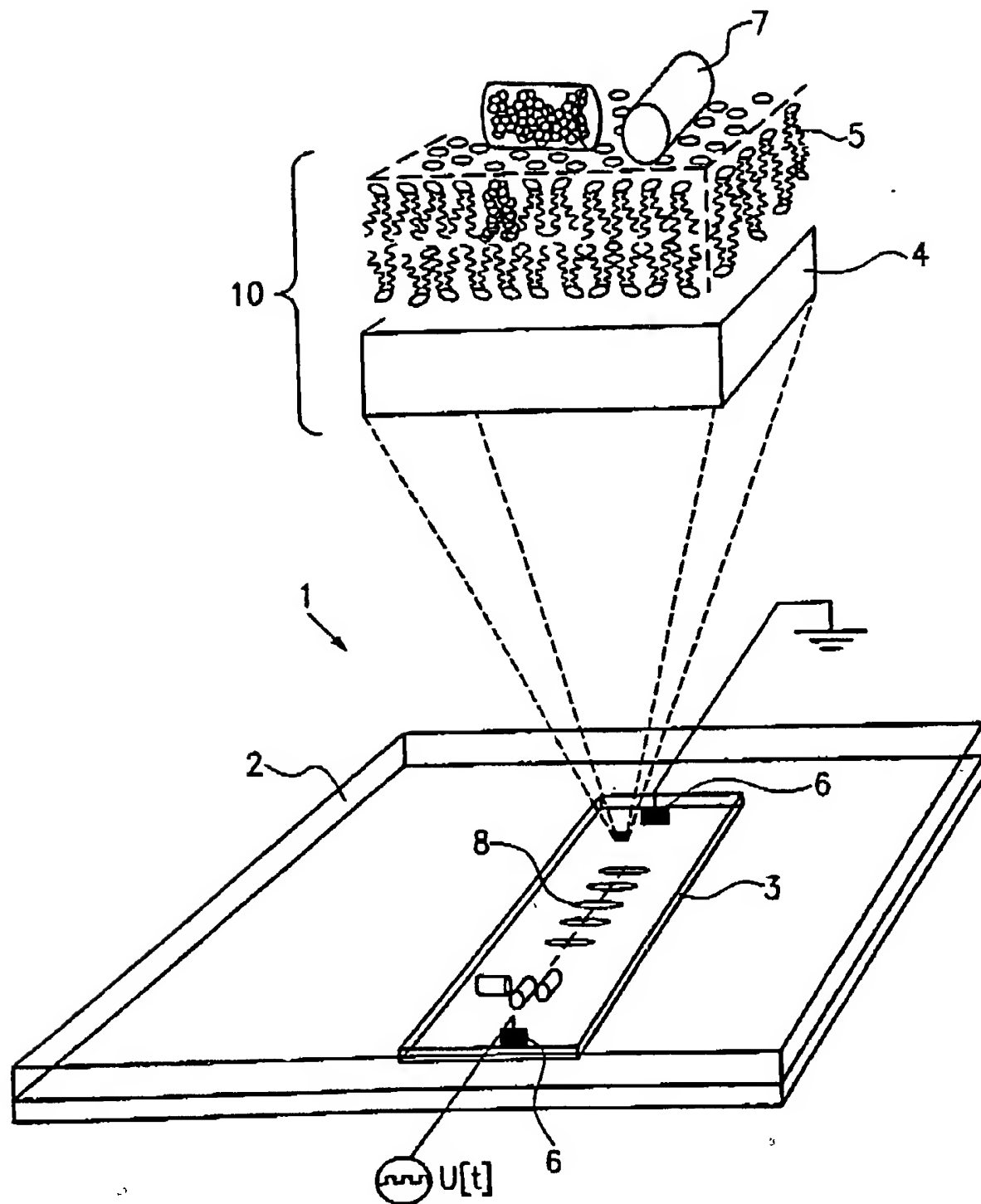


FIG. 1

ERSATZBLATT (REGEL 26)



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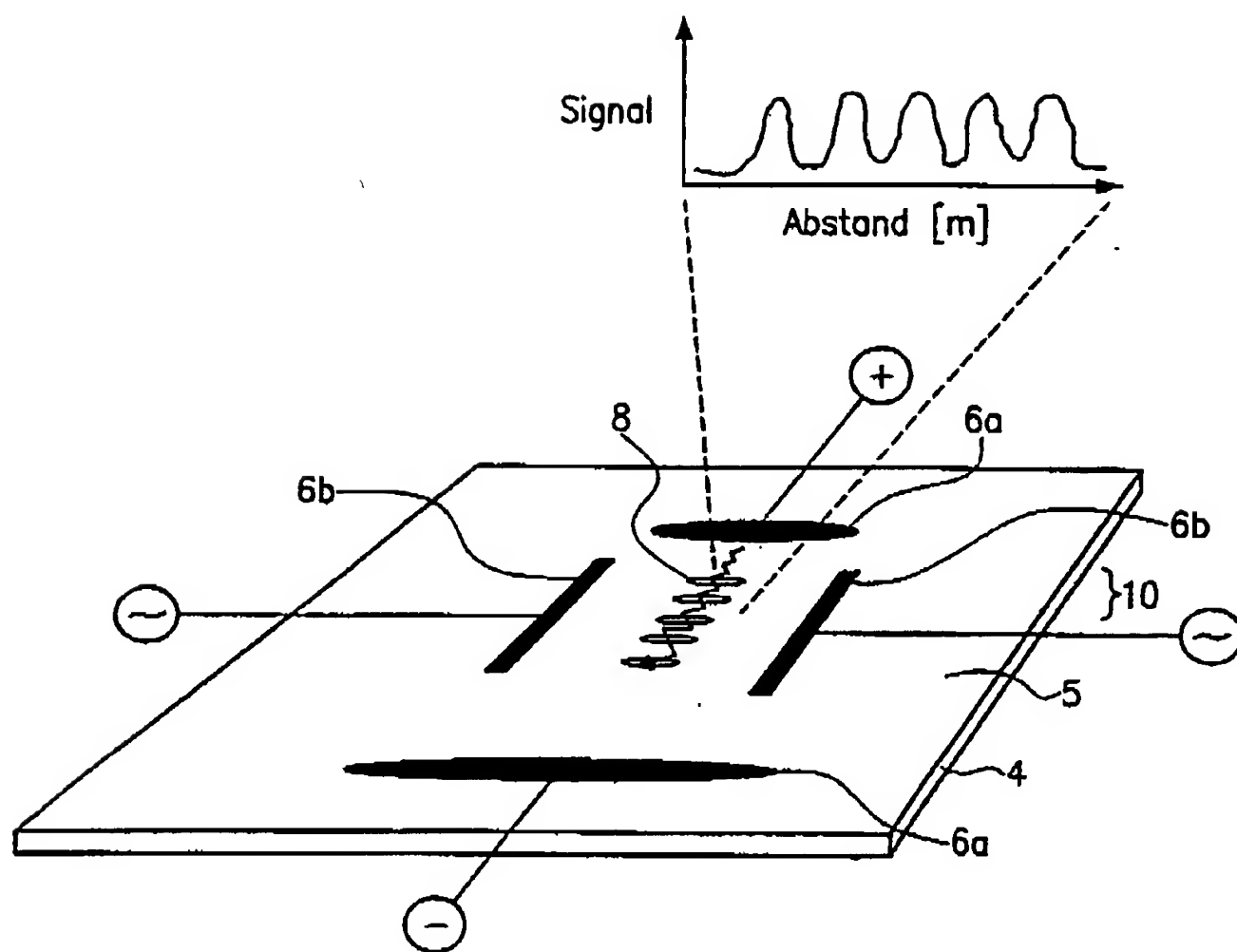


FIG. 2

ERSATZBLATT (REGEL 26)





## DECLARATION FOR UTILITY PATENT APPLICATION (37 CFR 1.63) POWER OF ATTORNEY

First Named Inventor: KAHL, et al. Attorney Docket Number GRUNP118  
 Application Number: 10/049,245 ☐ Declaration Submitted with Initial Filing  
 Filing Date: January 25, 2002 ☒ Declaration Submitted after Initial Filing  
 Group Art Unit:                      (surcharge (37 CFR 1.16 (e)) required)  
 Examiner Name:                     

## As a below named inventor, I hereby declare that:

My residence, mailing address, and citizenship are as stated below next to my name.

I believe I am the original, first, and sole inventor (if only one name is listed below) or an original, first, and joint inventor (if plural names are listed below) of the subject matter that is claimed and for which a patent is sought on the invention entitled:

A METHOD AND APPARATUS FOR THE SEPARATION OF PARTICLES, ESPECIALLY OF MACROMOLECULES, BY ELECTROPHORESIS  
 the specification of which

☐ is attached hereto.

OR  
☒ was filed on 1/25/2002 (MM/DD/YYYY) as U.S. Application Number or PCT International Application  
 Number 10/049,245 and was amended on                      (MM/DD/YYYY) (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment specifically referred to above.

I acknowledge the duty to disclose information that is material to patentability as defined in 37 CFR 1.56, including for continuation-in-part applications, material information that became available between the filing date of the prior application and the national or PCT international filing date of the continuation-in-part application.

I hereby claim foreign priority benefits under 35 U.S.C. 119(a)-(d) or (f), or 365(b) of any foreign application(s) for patent, inventor's or plant breeder's rights certificate(s), or 365(a) of any PCT international application that designated at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application for patent, inventor's or plant breeder's rights certificate(s), or any PCT international application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application Number(s)	Foreign Filing Date (MM/DD/YYYY)	Country	Priority Not Claimed	Certified Copy Attached?	
				YES	NO
PCT/EP00/07206	07/26/2000	PCT	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>
199 35 028.0	07/26/1999	Germany	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>

I hereby appoint Thomas M. Champagne (Reg. No. 36,478); Paul W. Koda (Reg. No. 44,191); and George F. Wallace (Reg. No. 45,286) as attorneys to prosecute the application identified above, and to transact all business in the United States Patent and Trademark Office connected therewith.

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the specification of which

☐ is attached hereto.

☒ was filed on 1/25/2002 (MM/DD/YYYY) as U.S. Application Number or PCT International Application Number 10/049,245 and was amended on \_\_\_\_\_ (MM/DD/YYYY) (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment specifically referred to above.

I acknowledge the duty to disclose information that is material to patentability as defined in 37 CFR 1.56, including for continuation-in-part applications, material information that became available between the filing date of the prior application and the national or PCT international filing date of the continuation-in-part application.

I hereby claim foreign priority benefits under 35 U.S.C. 119(a)-(d) or (f), or 365(b) of any foreign application(s) for patent, inventor's or plant breeder's rights certificate(s), or 365(a) of any PCT international application that designated at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application for patent, inventor's or plant breeder's rights certificate(s), or any PCT international application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application Number(s)	Foreign Filing Date (MM/DD/YYYY)	Country	Priority Not Claimed	Certified Copy Attached?	
				YES	NO
PCT/EP00/07206	07/26/2000	PCT	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>
189 35 028.0	07/26/1999	Germany	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>

I hereby appoint Thomas M. Champagne (Reg. No. 36,478); Paul W. Koda (Reg. No. 44,191); and George F. Wallace (Reg. No. 45,286) as attorneys to prosecute the application identified above, and to transact all business in the United States Patent and Trademark Office connected therewith.

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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